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Optimization of insulin-loaded nanoparticle-hydrogel delivery systems to accelerate wound healing

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Introduction

Chronic wounds (CW) is a severe health problem affecting patients worldwide and commonly associated to lack of growth factors in the wound bed. This presents a challenging problem, since current therapies are not highly effective, presenting an extended healing time, high recurrence rates and risk of amputations [1]. Insulin is one of the cheapest growth factors available, stimulating wound healing (WH) by promoting growth of granulation tissue and reepithelialisation, improving angiogenesis and reducing healing time [2]. However, the harsh proteolytic effect in the wound bed requires a delivery system (DS) able to protect insulin from degradation [3].

Our aim was to develop an insulin-loaded multifunctional nanoparticle (Np) hydrogel DS to accelerate WH and reduce frequency of dressing changes, improving life quality of patients and decreasing economic burden in healthcare systems.

Methods

Insulin-loaded chitosan-coated PLGA Np were produced by w/o/w double-emulsion technique [3] and embedded in hydrogels obtained by freeze-thawing. The DS was optimized by quality-by-design, varying chitosan (0.25%, 0.5%, 0.75%), alginate (5%, 7.5%, 10%), glycerine (1%, 1.5%, 2%), and the number of freeze-thawing cycles (1, 2, 3). Np were characterized by DLS and SEM, and hydrogels by rheology. Insulin structure was evaluated by FTIR, CD, and fluorescence spectroscopy.

Results and Discussion

Quality by design approach revealed an optimum hydrogel formulation. The hydrogel had good rheologic properties for skin application (Table 1). The Np showed particle size increase with the increase of chitosan concentration, and the change of the ZP into positive values shows the effective chitosan coating. Freeze-thawing ratio showed little aggregation of Np after freeze-thawing cycle. CD and FTIR results revealed that the insulin structure was preserved upon encapsulation and production of the hydrogel, with thioflavin assay results showing that there was no fibrillation of insulin - no insulin denaturation. SEM images showed that the Np incorporated into the hydrogel, maintaining its features with no relevant signs of particle aggregation.

The natural polymers used are multifunctional with chitosan promoting angiogenesis and mucoadhesion to the wound [4], and alginate stimulating inflammatory signals starting the WH process [5]. The developed multifunctional DS allows a sustained insulin delivery, protecting its stability and bioactivity. This prolongs residence time of insulin in wounds and may accelerate WH.

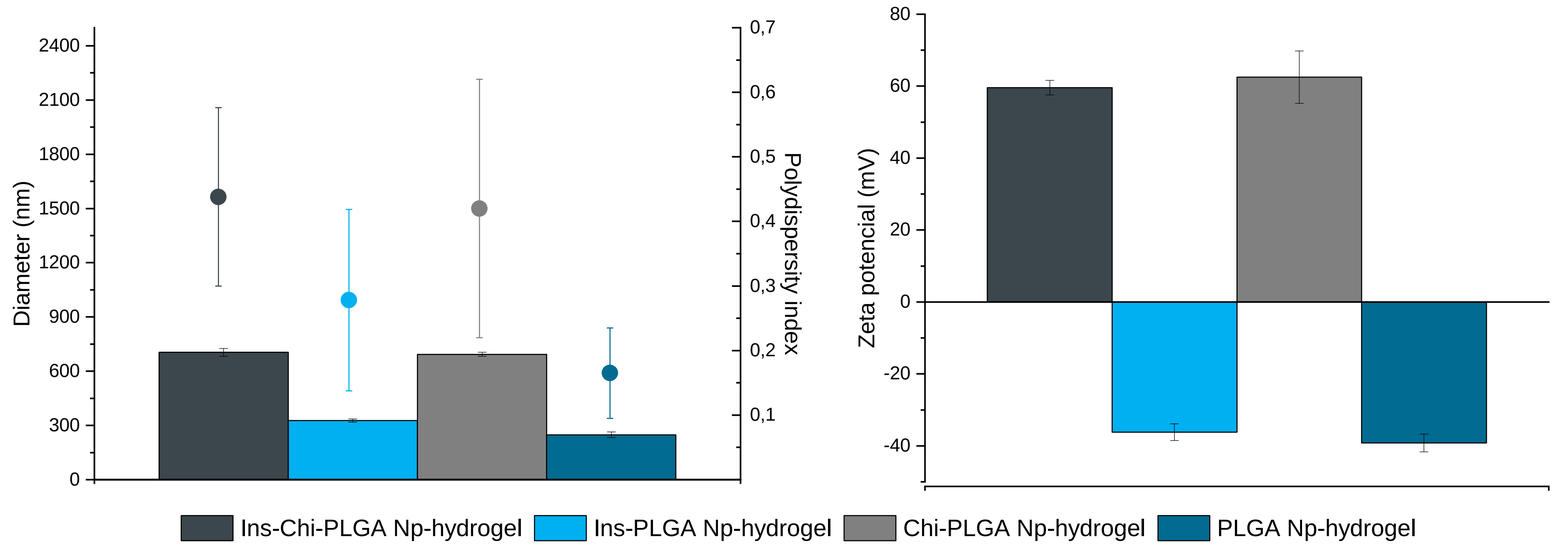

Figure 1: Mean particle size (left bars), polydispersity index (interval plot) and zeta potential (right bars) characterization of insulin-loaded chitosan-coated PLGA Np hydrogel, insulin-loaded PLGA Np hydrogel, chitosan-coated PLGA Np hydrogel and PLGA Np hydrogel.

Table 1: Freeze-thawing ratio, Water content and viscosity.

Formulation	Freeze-Thawing Ratio	Water content (%)	Viscosity (cP)
Ins-Chi-PLGA Np-hydrogel	0.97 ± 0.2	89.2 ± 0.1	6168 ± 1811
Ins-PLGA Np-hydrogel	1.56 ± 0,4	88.6 ± 0.2	4052 ± 521
Chi-PLGA Np-hydrogel	1.41 ± 0.3	89.3 ± 0.2	5801 ± 1688
PLGA Np-hydrogel	1.68 ± 0.3	88.7 ± 0.3	4819 ± 789

Table 2: Thioflavin T fluorescence assay.

Formulation	Thioflav
Ins-Chi-PLGA Np-hydrogel	-0.31 ± 0.12
Ins-PLGA Np-hydrogel	-0.33 ± 0.19
Chi-PLGA Np-hydrogel	-0.19 ± 0.24
PLGA Np-hydrogel	-0.23 ± 0.16
Positive Control	92.7 ± 0.34
Negative Control	0.029 ± 0.27

Conclusion

The method tested allowed for successful Np incorporation in the hydrogel, keeping their stability. The hydrogel also showed good rheological properties for topical application. The encapsulation of insulin revealed to be a good strategy to stabilize insulin structure without denaturation.

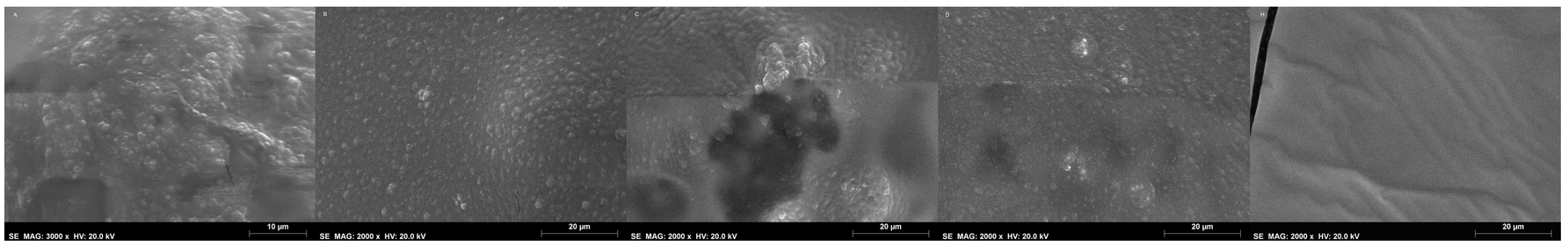

Figure 2: Nanoparticles-hydrogel SEM images. From left to right: insulin-loaded chitosan-coated PLGA Np hydrogel, insulin-loaded PLGA Np hydrogel, chitosan-coated PLGA Np hydrogel, PLGA Np hydrogel and blank hydrogel.

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